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ARRANGEMENT FOR EXAMINING MICROSCOPIC PREPARATIONS WITH A SCANNING MICROSCOPE, AND ILLUMINATION DEVICE FOR A SCANNING MICROSCOPE

CROSS REFERENCE TO RELATED APPLICATIONS

This invention claims priority of the German patent application DE 100 30 013.8 and 101 15 509.3 which is incorporated by reference herein.

FIELD OF THE INVENTION

The invention concerns an arrangement for examining microscope preparations with a scanning microscope, In particular, the invention concerns an arrangement for examining microscopic preparations with a scanning microscope that comprises a laser and an optical means which images the light generated by the laser onto a specimen that is to be examined. The scanning microscope can also be configured as a confocal microscope.

The invention furthermore concerns an illumination device for a scanning microscope.

BACKGROUND OF THE INVENTION

In scanning microscopy, a specimen is scanned with a light beam. Lasers are often used as the light source for this purpose. An arrangement having a single laser which emits several laser lines is known, for example, from EP 0 495 930, "Confocal microscope system for multi-color fluorescence." Mixed-gas lasers, in particular ArKr lasers, are usually used at present for this purpose.

Diode lasers and solid-state lasers are also in use.

US Patent 5,161,053 entitled "Confocal microscope" discloses a confocal microscope in which light of an external light source is transported with the aid of a glass fiber to the beam path of the microscope and the end of the glass fiber serves as a point light source, so that a mechanical stop is superfluous.

The emission spectrum of lasers is confined to a narrow wavelength range, so that for simultaneous multiple-line excitation, the light of several lasers must be combined into one illumination beam.

The gas lasers usually used as multiple-line lasers are very complex and expensive. They moreover require a great deal of maintenance, making them difficult to use continuously in many microscopy applications.

SUMMARY OF THE INVENTION

It is the object of the invention to create a scanning microscope which makes possible specimen examination with several spectral lines without requiring the use of multiple-line lasers or more than one laser.

The aforesaid object is achieved by a scanning microscope comprising:
a laser, an optical means for imaging light generated by the laser onto a specimen and an optical component positioned between the laser and the optical means, wherein the light generated by the laser passes through the optical component whereby the optical component spectrally spreads the light passing through.

A further object of the invention is to create an illumination device for a scanning microscope which provides an illumination encompassing a numerous selectable spectral regions.

The aforesaid object is achieved by an illumination device comprising a laser which has a light exit opening, an optical component made of photonic band-gap material which is mounted at the light exit opening.

It a further object of the invention to create a confocal scanning microscope which makes possible specimen examination with several spectral lines without requiring the use of multiple-line lasers or more than one laser.

The aforesaid object is achieved by a confocal scanning microscope comprising: a laser, an optical means for imaging light generated by the laser onto a specimen, a detector for receiving light coming from the specimen, an optical component positioned between the laser and the optical means, wherein the light generated by the laser passes through the optical component, whereby the optical component spectrally spreads the light passing through and an illumination pinhole through which the specimen is illuminated by the light emerging from the optical component.

It a further object of the invention to create a scanning microscope which makes possible specimen examination with several spectral lines without requiring the use of multiple-line lasers or more than one laser and which is realized in a simple and cost effective way.

The aforesaid object is achieved by a scanning microscope comprising: a pulsed laser, an optical means for imaging light generated by the pulsed laser onto a specimen and a tapered light-guiding fiber positioned between the pulsed laser and the optical means, wherein the light generated by the pulsed laser passes through the tapered light-guiding fiber whereby the tapered light-guiding fiber spectrally spreads the light passing through.

The optical component in the form of a photonic band-gap material has the advantage that the optically nonlinear construction of the fiber causes a short laser pulse to be spread out, thus creating a spectrally broad, continuous light spectrum. A "photonic band-gap material" is a microstructured, transparent material. It is possible, usually by assembling various dielectrics, to impart to the resulting

crystal a band structure which is reminiscent of the electron band structure of semiconductors.

The technology has recently also been implemented in light-guiding fibers. The fibers are manufactured by drawing out structured glass tubes. The fibers have a particular underlying structure: small capillaries are left open in the fiber direction, spaced approximately 2-3 μm apart and with a diameter of approx. 1 μm, and usually filled with air. No capillaries are present in the center of the fiber. These kinds of fibers are known as "photon crystal fibers," "holey fibers," or "microstructured fibers."

Photon crystal fibers can be used to produce a continuous spectral distribution over the entire visible wavelength region. This is done by coupling the light of a short-pulse laser into the fiber. The optically nonlinear construction of the fiber causes the frequency spectrum of the laser to spread out, creating a spectrally broad, continuous spectrum.

It is an other advantage of the invention to provide an embodiment which is simple an cost effective to realize. The optical component is a light-guiding fiber with a fiber core, wherein the fiber has a thinning provided on a part of the fiber. Light-guiding fibers of that kind are known as "tapered fibers". Preferable, the light-guiding fiber has an overall length of one meter an the thinning is provided over a length of 30 mm to 90 mm. The diameter of the fiber is 150 μm and diameter of the fiber core is approx. 8 μm. A the thinning the diameter of the fiber is reduced to approx. 2 μm. Consequently the diameter of the fiber core is the range of a few nanometers.

For use in microscopy, it is important to implement means for wavelength selection and for light output stabilization. A fiber laser of this kind can therefore advantageously be combined with acoustooptical or electrooptical tunable filters (AOTFs), acoustooptical or electrooptical deflectors (AODs), or acoustooptical or electrooptical beam splitters (AOBSs). These can be used not only for wavelength

selection but also to block out detected light (our German application DE 199 06 757 A1: "Optical arrangement").

In confocal microscopy in particular, the fiber exit end can be used as a point light source, thus making the use of an excitation aperture superfluous. With a configuration of this kind, it would be particularly advantageous for the fiber end itself to have a partially reflective coating, so that this partial reflector forms a resonator end mirror.

Further embodiments make provision for apparatuses to compensate for light output fluctuations. It is possible, for example, to incorporate a control loop for light output stabilization, which measures the light output in the beam path of the microscope in parasitic fashion, and maintains a constant specimen illumination light output by, for example, varying the pumping light output or with the aid of an acoustooptical or electrooptical element. LCD attenuators could also be used for this purpose.

A further advantage of the invention is that if the illumination device is already appropriately configured, it supplies several spectral regions for illumination. The laser which constitutes the illumination device for a scanning microscope has an optical component attached at the light exit opening. The optical component is made of photonic band-gap material. The photonic band-gap material can also be configured as a light-guiding fiber.

BRIEF DESCRIPTION OF THE DRAWINGS

The subject matter of the invention is schematically depicted in the drawings and is described below with reference to the Figures, in which:

FIG. 1 shows an arrangement according to the present invention with a confocal microscope;

FIG. 2 shows an arrangement in which an illumination pinhole has been omitted,

FIG. 3 shows an arrangement with light output stabilization,

Fig. 4 shows an embodiment of the optical component and

Fig. 5 shows a further embodiment of the optical component.

DETAILED DESCRIPTION OF THE INVENTION

FIG. 1 shows a confocal microscope that uses an optical component 3 to spread out a laser pulse generated by a pulsed laser 1. Pulsed laser 1 defines a pulsed laser beam 2 that is directed through optical component 3. Optical component 3 is a photonic band-gap material. What emerges from optical component 3 is a spectrally broad-band illuminating light 4 that is imaged by a first optical system 5 onto an illumination pinhole 6 and then strikes a beam splitter 7. From beam splitter 7, the spectrally broad-band illuminating light 4 passes to a second optical system 8 which generates a parallel light beam 4a that strikes a scanning mirror 9. Scanning mirror 9 is followed by several optical systems 10 and 11 which shape light beam 4a. Light beam 4a passes to an objective 12, by which it is imaged onto a specimen 13. The light reflected or emitted from the specimen defines an observation beam path 4b. The light of observation beam path 4b passes once again through second optical system 8, and is imaged onto a detection pinhole 14 that sits in front of a detector 15. Optical component 3 makes it possible to generate the laser light necessary for the examination of specimen 13 in accordance with the desired spectrum.

The exemplary embodiment depicted in FIG. 2 shows a confocal microscope in which illumination pinhole 6 has been omitted. All elements identical to the elements of FIG. 1 are labeled with the same reference characters. In this exemplary embodiment, an acoustooptical tunable filter (AOTF) 16, which is connected to a corresponding AOTF drive system 17, is used instead of first optical system 5. Since optical component 3 can generate a broad-band illuminating light 4, it is necessary to provide means for wavelength selection and for light output stabilization. Advantageously, acoustooptical or electrooptical tunable filters (AOTFs) can be combined with acoustooptical or electrooptical deflectors (AODs) and acoustooptical or electrooptical beam splitters (AOBSs). These can be used not only for wavelength selection but also to block out detected light. Also associated with AOTF 16 is a beam dump 18 which intercepts the

unused spectral portions of the illuminating light in order to prevent unnecessary disturbance of the scanning microscope.

A further embodiment of the invention is depicted in FIG. 3. Here a light-guiding fiber 20 made of the photonic band-gap material is used instead of optical component 3. From pulsed laser 1, pulsed laser beam 2 is coupled via an optical system 19 into an entrance end 20a of light-guiding fiber 20. Since light-guiding fiber 20 is constructed from the photonic band-gap material, a spectrally spread laser pulse emerges from exit end 20b and is coupled out via an optical system 21. Before the spectrally spread laser pulse strikes illumination pinhole 6, spectral filtering is performed. For that purpose, several color filters 24 are arranged on a turret 23. Turret 23 can be rotated by a motor 22, so that the corresponding color filters 24 can be introduced into the beam path. Also conceivable is a linear arrangement of color filters 24, in which case color filters 24 are moved by means of a linear motion into an illumination beam path 50. After illumination pinhole 6, illumination beam path 50 is comparable to the beam path of FIG. 1. As already mentioned in FIG. 1, beam splitter 7 deflects the light onto scanning mirror 9. A portion of the light passes through beam splitter 7 and defines a lost beam path 50a. This portion of the light is lost for observation or measurement purposes. For this reason, there is provided in lost beam path 50a a detector 25 which determines the lost light and ascertains therefrom an electronic variable that is conveyed via a line 30 to an electronic control system 26. Electronic control system 26 is connected via a further line 32 to pulsed laser 1. Electronic control system 26 regulates the intensity of pulsed laser 1, via line 32, in such a way that a constant light output always strikes specimen 13. For example, a control loop can be provided for light output stabilization, in such way that it measures the light output in the beam path of the microscope in parasitic fashion, and maintains a constant specimen illumination light output by, for example, varying the pumping light output or with the aid of an acoustooptical or electrooptical element. LCD attenuators could also be used for this purpose.

Fig. 4 shows a schematic representation of the optical component 3. The optical component 3 is a conventional light-guiding fiber 51, which has a overall diameter of 125 μm and the fiber core 52 has a diameter of 6 μm. In the area of a thinning 53, which is approx. 300 mm long, the overall diameter of the light-guiding fiber 51 is reduced 1.8 μm. In this area the diameter of the fiber core 52 is in the range of a few nanometers.

Fig. 5 shows a further embodiment of the optical component 3. The optical component 3 is a microstructured optical element. It consists of photonic band gap material, which has a special honeycombed microstructure 54. The honeycombed structure 54 that is shown is particularly suitable for generating broadband light. The diameter of the glass inner cannula 55 is approximately 1.9 μm. The inner cannula 55 is surrounded by glass webs 56. The glass webs 56 form honeycombed cavities 57. These micro-optical structure elements together form a second region 58, which is enclosed by a first region 59 that is designed as a glass cladding.

The present invention was described with reference to particular embodiments. It is self-evident, however, that changes and modifications can be made without leaving the spirit and the scope of the claims.